US ERA ARCHIVE DOCUMENT

DATA EVALUATION RECORD

- CHEMICAL: Mancozeb. 1, Shaughnessey No. 14504.
- TEST MATERIAL: Mancozeb (Dithane® M-45 Fungicide); Lot No. 2. E5803; TD No. 89-070; 80.1% active ingredient; a yellow powder.
- STUDY TYPE: Avian Reproduction Study. 3, Species Tested: Mallard duck (Anas platyrhynchos).
- CITATION: Beavers, J.B., G. Marselas, G.J. Smith, and M.J. đ, Jaber, 1991. Mancozeb: A One-Generation Reproduction Study with the Mallard (Anas platyrhynchos). Prepared by Wildlife International Ltd., Easton, MD. Laboratory Project No. 129-144. Submitted by Rohm and Haas Company, Spring House, PA. EPA MRID No. 419484-01.
- REVIEWED BY: 5.

Signature: Signature: 8-31-92 Dennis J. McLane Wildlife Bioligist Date: Ecological Effects Branch Ecological Fate and Effects Division

ORC

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APPROVED BY: 6.

> Les Touart Section Chief

Ecological Effects Branch

Date: Environmental Fate and Effects Division

reductions, the NOEC was 125 ppm ai.

CONCLUSIONS: This study is scientifically sound but does 7. net fulfill 5 the guideline requirements for an avian reproduction study. Nominal dietary concentrations of 10, 50, 125, and 1000 ppm ai had no effects upon mortality, behavior, adult body weight, egg shell thickness, or food consumption in mallards during the 18-week exposure period. However, the following reproductive parameters were significantly reduced at 1000 ppm ai: egg production, early and late embryo viability, hatchability, and offspring weight at hatch and 14-days of age. Based on these

- RECOMMENDATIONS: N/A. 8.
- BACKGROUND: Required as a result of the Mancozeb Registration Standard

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- 5, REVIEWED BY:

Kimberly Rhodes, M.S. Associate Scientist KBN Engineering and Applied Sciences, Inc.

APPROVED BY: 6.

> Michael L. Whitten, M. & Wildlife Toxicologist/ KBN Engineering and Applied Sciences, Inc.

Henry T. Craven, M.S. Supervisor, EEB/ÉFED USEPA

Signature: Annexy Ander

Date: 4/4/92

Signature: Midal L. White

6/4/92 Date:

Signature

Date:

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RECOMMENDATIONS: N/A. 8.

29 45

reductions, the NOEC was 125 ppm ai.

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

- Test Animals: The birds used in the test were penreared mallards (Anas platyrhynchos) approaching their
 first breeding season and were purchased from Whistling
 Wings, Hanover, Illinois. The birds were acclimated to
 the facilities for 8 weeks prior to test initiation.
 All birds were from the same hatch and were
 phenotypically indistinguishable from wild birds. At
 test initiation, all birds were examined for physical
 injuries and general health. Birds that did not appear
 healthy were discarded. The birds were 25 weeks of age
 at test initiation.
- B. Dose/Diet Preparation/Food Consumption: Test diets were prepared by mixing the test material into a pre-mix which was used for weekly preparation of the final diet. The control diet and four test concentrations (10, 50, 125, and 1000 ppm) were prepared weekly and presented to the birds on Thursday of each week. When necessary, additional feed was prepared. Each of the five groups of adult birds was fed the appropriate diet from test initiation until terminal sacrifice. Dietary concentrations were adjusted for purity of the test substance, and are presented as ppm of active ingredient.

Basal diet for adult birds and their offspring was formulated by Agway, Inc. The composition of the diet was presented in the report. The test substance was not mixed into the diet of the offspring. Food and water were supplied ad libitum during acclimation and during the test for adults and offspring.

Six samples from the control and each treatment concentration were collected on day 0 of week 1 to determine the homogeneity of the test material in the diet. Stability samples were collected from week 1 samples stored at ambient conditions for 7 and 14 days. Verification samples were collected immediately after mixing and placed in the study room where they were exposed to ambient conditions for a period of 7 days. On day 7, these samples were placed in a freezer and stored frozen until shipped frozen on dry ice to the analytical laboratory. Verification samples were collected initially and during weeks 2, 3, 4, 8, 12 and 16. Samples were analyzed by Enviro-Bio-Tech, LTD.

C. <u>Design</u>: The birds were randomly distributed into five groups as follows:

Dithane® M-45 Techn: Nominal	ical	Number	Rirde	Per Pen
Concentration		of Pens	Males	
Control (0 ppm) 10 ppm ai	منتر *	16 16	1	1
50 ppm ai		16	ī	ī
125 ppm ai		16	1	1
1000 ppm ai		16	1	1

Treatment levels were based upon known toxicity data and consultation with the client. Adult birds were identified by individual leg bands. The primary phases of the study and their approximate durations were as follows:

- 1. Acclimation 8 weeks
- 2. Pre-photostimulation 8 weeks
- 3. Egg laying 10 weeks
- 4. Post-adult sacrifice (final incubation, hatching, 14-day offspring rearing period) 6 weeks.
- D. <u>Pen Facilities</u>: Adult birds were housed indoors in pens constructed of wire grid and sheeting. Pens measured approximately 75 x 90 x 45 cm high. The average temperature in the adult study room was 19.8 ±2.4°C (SD) with an average relative humidity of 56 ±21% (SD).

The photoperiod during acclimation and during the first 8 weeks of the study was eight hours of light per day. The photoperiod was increased to 17 hours of light per day at the beginning of week 9 and was maintained at that level until sacrifice of adult birds. The birds were exposed to approximately 130 lux of illumination throughout the study.

E. Adult Observations/Gross Pathology: All adult birds were observed at least once daily throughout the study for signs of toxicity or abnormal behavior. All birds that died during the study were necropsied. As soon as practical after the death of the bird, the pen mate was sacrificed and necropsied. At study termination, all surviving birds were sacrificed and necropsied. Adult birds were weighed at test initiation, during weeks 2, 4, 6, 8, and at study termination. Food consumption for a 7-day period was determined for each pen every week throughout the study.

Eggs/Eggshell Thickness: Eggs were collected daily from F. all pens, marked according to pen of origin, and washed to prevent pathogen contamination. The eggs were then stored at 13.0 \pm 1.6°C (SD) and a mean relative humidity of 70% until incubated. At weekly intervals, eggs were removed from the storage room and candled. Cracked or abnormal eggs were discarded. All eggs that were not cracked, abnormal or used for egg shell thickness measurements were placed in an incubator at 37.4 ±0.1°C (SD) and 53% relative humidity. Eggs were candled again on day 14 of incubation to determine embryo viability and on day 21 to determine embryo survival. All eggs were turned automatically while in the incubator. eggs were placed in a hatcher on incubation day 24. The average temperature in the hatcher was 37.0 \pm 0.4°C (SD) with an average relative humidity of 70%.

Weekly throughout the egg laying period, one egg was collected, when available, from each of the odd numbered pens during the odd numbered weeks, and from each of the even numbered pens during the even numbered weeks. These eggs were opened at the equator, the contents removed, the shell washed thoroughly and allowed to dry for at least one week. The average thickness of the dried shell plus membrane was determined by measuring (to the nearest 0.005 mm) five points around the waist of the egg using a micrometer.

- G. Hatchlings: All hatchlings and unhatched eggs were removed from the hatcher on day 27 or 28 of incubation. The average body weight of the hatchlings by pen was then determined. Hatchlings were toe and web clipped for identification by pen of origin and then placed in brooding pens until 14 days of age. Each brooding pen measured 72 cm x 90 cm x 24 cm high, and was constructed of vinyl-coated wire mesh. Temperatures in the brooding compartment were approximately 38°C until the birds were 5 to 7 days of age, and 26°C thereafter. The photoperiod was maintained at 16 hours of light per day. Hatchlings were fed untreated diet. At 14 days of age, the average body weight by parental pen of all survivors was determined.
- H. Statistics: Upon completion of the study, Dunnett's method was used to determine statistically significant differences between the control group and each of the treatment groups. Sample units were the individual pens within each experimental group. Percentage data were examined using Dunnett's method following arcsine

transformation. The pens in which mortality occurred were not used in statistical comparisons of the data.

Each of the following parameters was analyzed statistically:

Adult Body Weight
Adult Feed Consumption
Eggs Laid of Maximum Laid
Eggs Cracked of Eggs Laid
Viable Embryos of Eggs Set
Live 3-Week Embryos of
Viable Embryos
Hatchlings of 3-Week
Embryos
Hatchlings of Eggs Set

Offspring's Body Weight
Hatchlings of Maximum Set
14-Day Old Survivors of
Maximum Set
14-Day Old Survivors of
Eggs Set
14-Day Old Survivors of
of Hatchlings
Egg Shell Thickness

12. REPORTED RESULTS

A. <u>Diet Analysis</u>: The results of the diet analyses are presented in Tables I and II (attached) of Appendix XII. Nominal and mean measured concentrations of freshly prepared diets were as follows:

Dith	ane® M-45 Technical	(ppm ai)
Nominal Concentration	Mean Measured Concentration	% of Nominal Concentration
0	0.00	,
10	8.60	86
50	46.30	93
125	117.00	94
1000	914.00	91

Average recovery-adjusted concentrations ranged from 96-101% of nominal values for homogeneity samples. Stability samples ranged from 82-89% after 7 days of storage under ambient conditions.

B. Mortality and Behavioral Reactions: There were no mortalities in the control, or in the 10, 50, or 125 ppm ai groups. One incidental mortality occurred in the 1000 ppm ai treatment group.

Necropsy results of the mortality at 1000 ppm ai and sacrificed birds were included in the report. All lesions observed in the one dead bird and sacrificed birds were considered to be unrelated to treatment.

No overt signs of toxicity were observed at any concentration. Incidental clinical signs noted in the control and various treatment groups included slight lethargy, a ruffled appearance, loss of coordination, lower limb weakness, walking on toes, and swollen sinuses.

C. Adult Body Weight and Food Consumption: When compared to the control group, there were no statistically significant differences in body weights at any concentration tested (Table 1, attached).

Due to excessive wastage by some birds, feed consumption was variable between pens. There were no apparent treatment related effects upon feed consumption among birds at any test level (Table 2, attached).

D. <u>Reproduction</u>: There were no statistically significant differences in reproductive parameters between the control and the 10, 50, or 125 ppm ai treatment groups.

In the 1000 ppm ai group, statistically significant (p<0.01) reductions in eggs laid, viable embryos, live 3-week embryos, hatchability and the number of hatchlings and 14-day old survivors were observed. The percentage of cracked eggs and survival of hatchlings to 14 days of age were not affected in the 1000 ppm ai treatment group (Tables 3 & 3A, attached).

- E. Egg Shell Thickness: There were no significant differences in egg shell thickness between the control and any test concentration (Table 4, attached).
- F. Offspring Body Weight: When compared to the control group, there was no significant difference in offspring body weight at any concentration. However, while not statistically significant, reductions in the body weight of hatchlings were observed at the highest treatment level (1000 ppm ai). There was a statistically significant (p<0.05) reduction in the body weight of 14-day old survivors at 1000 ppm ai. (Tables 5 & 5A, attached).
- The no-observed-effect concentration for mallards exposed to Dithane® M-45 was 125 ppm ai, based on treatment related effects on reproductive performance observed at 1000 ppm ai.

The report stated that the study was conducted in conformance with Good Laboratory Practice regulations (40 CFR Part 160). Quality assurance audits were conducted during the study and the final report was signed by the Quality Assurance Auditor of Wildlife International Ltd.

14. Reviewer's Discussion and Interpretation of the Study:

A. <u>Test Procedure</u>: The test procedures were in accordance with Subdivision E - Hazard Evaluation: Wildlife and Aquatic Organisms, ASTM, and SEP guidelines except for the following deviations:

Eggs were stored at a temperature of approximately 13°C and a relative humidity of 70%; 16°C and 65% are recommended.

The bird were on the treated diet for only 8 weeks rather than the required 10 weeks p; rior to onset of egg laying.

The dose levels were spaced by a factor of five.

The photoperiod during the first 8 weeks of the study was 8 hours per day; guidelines recommend 7 hours or light per day during this period.

Behavioral observations of offspring were not reported.

Observations on food palatability were not reported.

All eggs were transferred to the hatcher on day 24. The guidelines recommend the transfer on day 23.

A recovery period (exposure to basal diet only) was not added to the treatment phase of the study.

B. <u>Statistical Analysis</u>: Statistical procedures differed from recommended methods. Specifically, there is no basis for transforming the number of eggs laid and the number of hatchlings to percentile values of the maximum number of eggs laid or set in any test group.

Statistical analyses of study parameters were performed using EEB's Birdall computer program (see attached SAS instuctions). Comparison of results presented by the authors and reviewer are shown in Table A (attached). Offspring body weight (at hatch) was significantly lower at 1000 ppm than in the control; the authors noted the

reductions but their analyses found no significant differences between groups.

C. <u>Discussion/Results</u>: This study is scientifically sound but does not fulfill the guideline requirements for an avian reproduction study. Nominal dietary concentrations of 10, 50, 125, and 1000 ppm ai had no effects upon mortality, behavior, adult body weight, egg shell thickness, or food consumption in mallards during the 18-week exposure period. However, the following reproductive parameters were significantly reduced at 1000 ppm ai: egg production, early and late embryo viability, hatchability, and offspring weight at hatch and 14-days of age. Based on these reductions, the NOEC was 125 ppm ai.

The birds were not on test diet for the required ten weeks prior to the onset of egg laying. A ten week exposure may result in a lower NOEL.

D. Adequacy of the Study:

- (1) Classification: Supplemental.
- (2) Rationale: The birds were not on test diet for the required ten weeks prior to onset of egg laying. This may have resulted in a higher NOEL in this test, 500 0 95007 cgc 2/9/95
- (3) Repairability: This study can not be repaired.
- 15, COMPLETION OF ONE-LINER: Yes; Aug 28, 1992.

TABLE A. Reproductive Parameters of Mallard Duck Exposed to Dithane® M-45. Comparison of Results Presented by Authors and Reviewer.

PARAMETER	REVIEWER: LEVELS (PPM) SIGNIFICANTLY AFFECTED	AUTHORS: LEVELS (PPM) SIGNIFICANTLY AFFECTED
Eggs Laid	1000	1000
Eggs Cracked	NS	(%) NS
Eggs Set	1000	NR
Viable embryos	1000	1000
Live 3-week embryos	1000	1000
Hatchlings	1000	1000
14-day old survivors	1000	1000
Eggs cracked/eggs laid	NS	NS
Viable embryos/eggs set	NS	1000
Live 3-week embryos/viable	NS	1000
Hatchlings/3-week	NS	1000
14-day old survivors/hatch	NS	NS
Hatchlings/eggs set	1000 ¹	1000
14-day old survivor/eggs set	1000 ¹	1000
Male weights	NS	NS
Female weights	NS	NS
Egg shell thickness	NS	NS
Hatchling weight	1000	NS
14-day old survivor weight	1000	1000
Food consumption	NS	NS
NR = NOT REPORTED		

NS = NOT SIGNIFICANT

1= KBN analysis

BrookisAs

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PROGRAM EDITOR
Command ===>
NOTE: 87 line(s) included.
00001 OPTIONS LINESIZE=66; PAGESIZE=95; option mtrace;
00002 %MACRO CAL1 ; PROC GLM; CLASSES TRT;
00003 MODEL RESP=TRT;
00004 MEANS TRT/DUNCAN; RUN; %MEND;
00005 %MACRO CAL2 ;
00006 ARS=ARSIN(SQRT(Z));
00007 RESPONSE=ARS*(180/(22/7));
00008 PROC GLM; CLASSES TRT; MODEL RESPONSE=TRT; WEIGHT WT;
00009 MEANS TRT/DUNCAN; RUN; %MEND;
00010 %MACRO CAL3 ;
00011 PROC GLM;
00012 CLASSES TRT;
00013 MODEL POSTM=TRT PREM / SOLUTION;
00014 LSMEAN TRT / E STDERR PDIFF;
00015 MEAN TRT/DUNCAN;
00016 RUN; %MEND;
00017 %MACRO CAL4 ;
00018 PROC GLM;
00019 CLASSES TRT;
00020 MODEL POSTF=TRT PREF / SOLUTION;
00021 LSMEAN TRT / E STDERR PDIFF;
                                                               ZOOM=
```

```
PROGRAM EDITOR
Command ===>
00022 MEAN TRT/DUNCAN;
00023 RUN; %MEND;
00024 DATA T;
00025 INFILE 'A:birdall.dat';
00026 INPUT TRT$ EL EC ES VE LE NH HS THICK HATWT SURVWT FOOD
00027 PREM POSTM PREF POSTF;
00028 PROC PRINT; RUN;
00029 PROC SORT; BY TRT; RUN;
00030 PROC MEANS; BY TRT; RUN;
00031 DATA TEMPA; SET T; RESP=EL;
00032 TITLE '1. ANALYSIS OF EL DATA';
00033 TITLE2 ' ************* %CAL1;
00034 DATA TEMPB; SET T; RESP=EC;
00035 TITLE '2. ANALYSIS OF EC DATA';
00036 TITLE2 ' ************** %CAL1;
00037 DATA TEMPC; SET T; RESP=ES;
00038 TITLE '3. ANALYSIS OF ES DATA';
00040 DATA TEMPD; SET T; RESP=VE;
00041 TITLE '4. ANALYSIS OF VE DATA';
```

```
PROGRAM EDITOR
Command ===>
00022 MEAN TRT/DUNCAN;
00023 RUN; %MEND;
00024 DATA T;
00025 INFILE 'A:birdall.dat';
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```
00026 INPUT TRT$ EL EC ES VE LE NH HS THICK HATWT SURVWT FOOD
00027 PREM POSTM PREF POSTF;
00028 PROC PRINT; RUN;
00029 PROC SORT; BY TRT;
00030 PROC MEANS; BY TRT; RUN;
00031 DATA TEMPA; SET T; RESP=EL;
00032 TITLE '1. ANALYSIS OF EL DATA';
00034 DATA TEMPB; SET T; RESP=EC;
00035 TITLE '2. ANALYSIS OF EC DATA';
00037 DATA TEMPC; SET T; RESP=ES;
00038 TITLE '3. ANALYSIS OF ES DATA':
00039 TITLE2 ' ************* %CAL1;
00040 DATA TEMPD; SET T; RESP=VE;
00041 TITLE '4. ANALYSIS OF VE DATA';
ZOOM=
```

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PROGRAM EDITOR
Command ===>
00043 DATA TEMPE; SET T; RESP=LE;
00044 TITLE '5. ANALYSIS OF LE DATA';
00045 TITLE2 '
             ******************* %CAL1;
00046 DATA TEMPF; SET T; RESP=NH;
00047 TITLE '6. ANALYSIS OF NH DATA';
00049 DATA TEMPG; SET T; RESP=HS;
00050 TITLE '7. ANALYSIS OF HS DATA';
00052 DATA TEMPH; SET T; RESP=THICK;
00053 TITLE '8. ANALYSIS OF EGGSHELL THICKNESS DATA';
00054 TITLE2 * ************* * * * * * CAL1;
00055 DATA TEMPI; SET T; RESP=HATWT;
00056 TITLE '9. ANALYSIS OF HATCHLING WEIGHT DATA';
00058 DATA TEMPJ; SET T; RESP=SURVWT;
00059 TITLE '10. ANALYSIS OF 14-DAY SURVIVOR WEIGHT DATA';
00060 TITLE2 '
             ****************** %CAL1;
00061 DATA TEMPK; SET T; RESP=FOOD;
00062 TITLE '11. ANALYSIS OF FOOD CONSUMPTION DATA';
00063 TITLE2 ' *************** %CAL1;
```

```
PROGRAM EDITOR
Command ===>
00064 DATA TEMP1; SET T; WT=EL; Z=ES/EL; DROP EC VE LE NH HS;
00065 TITLE '12. ANALYSIS OF ES/EL DATA';
             00066 TITLE2 '
00067 DATA TEMP2; SET T; WT=ES; Z=VE/ES; DROP EL EC LE NH HS;
00068 TITLE '13. ANALYSIS OF VE/ES DATA';
00069 TITLE2 '
              ******* %CAL2;
00070 DATA TEMP3; SET T ; WT=VE; Z=LE/VE; DROP EL EC ES NH HS;
00071 TITLE '14. ANALYSIS OF LE/VE DATA';
00073 DATA TEMP4; SET T; WT=LE; Z=NH/LE; DROP EL EC ES VE HS;
00074 TITLE '15. ANALYSIS OF NH/LE DATA';
00075 TITLE2 ' *********************** %CAL2;
```

```
PROGRAM EDITOR
Command ===>
00085 DATA TEMP13; SET T;
00086 TITLE '19. COVARIATE ANALYSIS OF FEMALE BODY WEIGHT DATA';
00088
00089
00090
00091
00092
00093
00094
00095
00096
00097
00098
00099
00100
00101
00102
00103
00104
00105
                                                  ZOOM=
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PROGRAM EDITOR
                                                  male
Command ===>
NOTE: 159 line(s) included.
00001 0 46 0 40 34 34 30 29 0.333 33 284 172.444 1185 1312 1642, 1182
00002 0 56 1 51 48 48 40 39 0.394 37 315 173.722 1276 1378
00003 0 40 0 36 30 25
                        6 0.371 39 277 151.556 1215 1314
                      6
                                  38 292 183.778 1203 1171
                      2
                          2 . .
       5 0 4
               3 3
00005 0 39 0 35 34 34 20 19 0.363 34 280 139.167 1210 1222
00006 0 54 0 50 41 41 25 25 0.395 36 298 160.611 1408 1316
00007 0 27 0 24 23 23 17 17 0.366 37 271 173.167 1173 1165
00008 0 52 0 48 47 47 38 38 0.377 34 277 181.500 1237 1357
00009 0 37 0 34 28 26 16 15 0.360 33 277 159.444 1357 1278
00010 0 46 0 42 40 40 25 23 0.386 34 312 133.944 1177 1185
00011 0 53 1 48 47 47 19 19 0.398 35 273 202.056 1142 1182
00012 0 50 0 46 38 38 28 28 0.382 37 313 209.556 1350 1272
                        8 0.380 38 301 185.833 1250 1304
00013 0 44 2 38 36 36 8
00014 0 54 0 50 48 48 44 43 0.375 34 285 202.444 1289 1329
00015 0 35 0 32 31 31 20 20 0.390 36 301 226.889 1272 1301
                        9 0.392 39 302 149.889 1057 1129
00016 0 43 2 38 36 36
                      9
00017 1 51 0 47 45 44 35 34 0.362 36 274 159.833 1122 1091
00018 1 47 0 43 43 43 34 32 0.365 36 291 157.611 1250 1283
00019 1 49 0 45 42 42 15 14 0.394 38 288 171.500 1083 1131
00020 1 54 0 50 48 48 22 22 0.366 37 312 248.444 1359 1352
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	Identity of product inert ingredients.
	Identity of product impurities.
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	Description of quality control procedures.
	Identity of the source of product ingredients.
· · · · · · · · · · · · · · · · · · ·	Sales or other commercial/financial information.
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	The product confidential statement of formula.
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